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Full Length Research Paper

A study on the sero epidemiology of African horse sickness in three woredas of Sidama Zone, Hawassa, Ethiopia

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A cross-sectional study was conducted in the three woredas of Sidama zone, Southern Nations, Nationalities and Peoples' Region (SNNPRS) with the aim of determining the overall prevalence of African horse sickness (AHS) in equines and the effect of putative risk factors on the prevalence of AHS. A total of 230 equines (66 donkeys, 161 horses and 3 mules) were included. Serological examination was conducted using blocking enzyme linked immuno sorbent assay (blocking ELISA) revealing a point prevalence of 46.97, 13.66 and 33.33% AHS in donkeys, horses and mules, respectively. The overall prevalence of AHS in equines in Sidama zone of the three districts was 23.47%. The prevalence at Dale was 52.72 and 10.47% in Hula and 17.98%, respectively at Wonsho woreda'. Statistical analysis of the result showed significance difference in prevalence among donkeys, horses and mules ($P < 0.05$). The prevalence of 52.72% in midland and 14.28% in highland revealed statistically significant difference ($P < 0.05$). The difference observed between Dale and Hula, and Dale and Wonsho was seen to be statistically different, but no difference was seen between Wonsho and Hula. Among the hypothesized risk factors, agro ecology is the predominant risk for the prevalence of AHS and this was asserted by P-value ($P < 0.05$).

Key words: African horse sickness, enzyme linked immuno sorbent assay (ELISA), equines, sero-epidemiology, Sidama, prevalence.

INTRODUCTION

African horse sickness (Peste equina Africana, Peste equine) is an insect born viral disease characterized by severe pyrexia, wide spread hemorrhage and edematous exudations (OIE, 2008). AHS virus affects all species of Equidae family (horses, mules, donkeys and zebras) and transmitted by a biting midge belonging to the genus *Culicoides* (Maclachlan and Guthrie, 2010).

This devastating disease is endemic in sub-Saharan African (OIE, 2008). In the late 1980s, AHS spread out side its endemic region reaching Spain and Portugal (OIE, 2008; Ferhandey et al., 2012).

African horse sickness virus (AHSV) belongs to the family *Reoviridae*, and genus *Orbivirus* (OIE, 2008). Like other *Orbiviruses*, AHSV virions are double-stranded

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with their genomes composed of 10 double stranded RNA (dsRNA) segments (Cetre-Sossah et al., 2008). Nine serologically distinct AHS virus serotypes (AHSV-1 to AHSV-9) have been identified with the evidence of cross-neutralization among them (Cetre-Sossah et al., 2008; OIE, 2004).

The disease confined to sub-Saharan Africa although periodic epizootics have caused severe outbreaks of the disease outside enzootic regions like North Africa, Middle East, and Southern Europe (Rodriguez-Sanchez et al., 2008; Torrecuadrada et al., 2011). The nine known virus serotypes of AHSV have been isolated from clinical cases of the disease in Kenya (Binopal et al., 2013). The most important factor in the epizootology of AHS is the host.

The existence of the reservoir of infection is suggested by the fact that the disease passes from one season to another in a particular area. An outbreak mostly occurs during rainy season and quickly disappears during dry and cool periods before it appears when wet and warm weather returns.

Many factors can contribute to the poor performance of equines, among which viral diseases characterized by high morbidity and mortality rates are the first. Clinically, the disease is characterized by an acute pulmonary form, a cardiac form or sub-acute form, mixed form and a mild form know as horse sickness fever (Upadhyaya, 2011). Hence, African horse sickness is one of the viral diseases characterized by up to 95, 50 and 10% mortality rates in horses, mules and donkeys, respectively (OIE, 2008).

According to the Central Statistical Authority of Ethiopia (2009) there are 5.42 million donkeys, 1.78 million horses and 373,519 mules. It has the largest equine population, probably with the highest density per square kilometer in the world (Muluaem et al., 2012). An important but often overlooked aspect is that in most cases, donkeys and mules are reared by land less and marginal farmers and are the means of subsistence for millions in the least privileged parts of the world. These beasts of burden receive little care and are subjected to intensive work throughout their lives. Like most parts of the country, in Sidama zone as well, the attention given to these animals was very poor despite their enormous role in the economy and they are subjected to arrays of management constraints and diseases.

Among the reported diseases, African horse sickness has been known for years (Sidama Zone Agricultural and Rural Department (SZARD), 2011). However, to the authors' knowledge, in the last decades, no systemically collected information are available pertinent to the disease in the study area. Hence, the research work aimed to estimate the prevalence of African Horse sickness and to identify some of the hypothesized risk factors in three woredas' of Sidama zone, Hawassa, Ethiopia.

MATERIALS AND METHODS

Study area

This study was carried out in the Sidama zone of Southern Nations, Nationalities and Peoples Region (SNNPRs). The zones is located in the northern part of SNNPRs, with its capital town at Hawassa, which lies about 275 km south of Addis Ababa. Geographically, the zone lies between 4°27' and 8°30' N latitude and 39°1' E longitude [Sidama Zone Planning and Economic Development Department (SZPEDD), 2004]. Like most parts of Ethiopia, the relief configuration of Sidama ranges from very high mountains to lowland plains, where the altitude varies between 1001 and 3200 m above sea level (m.a.s.l.). It has an annual rainfall and temperature ranging from 960 to 1100 mm and 18.1 to 20°C, respectively (SZPEDD, 2004).

Study animals

The study animals were equines, namely: horses, donkeys and mules above 4 years of age and non-vaccinated for AHS were considered from two ecological zones of midland (Dale) and highland (Wonsho and Hula) of Sidama zone. A total of 230 equines were selected, of which 66 were females and 164 males. Most of the equines in the area are used for pack, race and cart pulling.

Study design

The study was an observational one made in two distinct agro ecological zones namely midland and highland of the study area. As animals of the same village are usually kept in a communal grazing land, list of pass of respective district was used to represent primary sampling unit (epidemiological unit) and individual animals (secondary unit) using two stage cluster sampling technique. To this effect, a cross sectional study design was employed. Blood samples were collected using plain vacutainer tube and information (data) relevant to the epidemiology was collected using semi-structured questionnaire format.

Sample size

A two-stage cluster sampling techniques was used to calculate the actual sample size having the following parameters predetermined: CL= 90%, desired level of precision =5%, expected total clusters prevalence and in between cluster variance (Thrusfield, 2005). Average number of equines per peasant association (n) is estimated to be ten (10), using the formula:

$$g = \frac{1.96^2(nVc + Pexp(1 - Pexp))}{nd^2} = 21$$

Where, n = herd size; Vc = in between cluster variance; D= desired level of precision; Pexp= expected prevalence; g = number of clusters needed. In order to get the total sample size,

$$T_s = \frac{1.96^2 * g * Pexp(1 - Pexp)}{gd^2 - 1.96^2 Vc} = 230$$

A total of 230 equines in 21 clusters were picked randomly

Table 1. Prevalence of AHS in the three districts

Locations	Equines		
	Number mined	Number seropositive	Prevalence (%) 95%CI
Dale	55	29	52.72 (39.10-66.35)
Hula	86	9	10.47 (3.86-17.07)
Wonsho	89	16	17.98 (9.84-26.11)
Total	230	54	23.47

considering 10 clusters from midland and 11 clusters from highland agro ecological zones.

Diagnostic test

Serum was collected from three selected sites and targeted species; to this effect, 10 ml of blood using non-heparinized vacutainer tube were collected and sera were harvested from the clotted blood that was kept for 30 min at 37°C (OIE, 2004). Each serum sample was given an identification code and kept at -20°C until assayed in the laboratory. At the National Veterinary Institute (NVI), ELISA was used to detect the presence of specific antibody against the AHS virus in the sera samples collected.

Test procedure

All reagents were brought to room temperature before use. 100 µl of diluted samples was dispensed into appropriate wells (dilution 1/5). Running the samples in duplicate was recommended. 100 µl of positive control was dispensed into two wells and 100 µl of negative control into two wells. The plate was covered and incubated for 1 h at 37°C and washed 5 times. Then, 100 µl/well of conjugate was added and incubated for 30 min at 37°C.

Again, it was washed 5 times. 100 µl/well of substrate solution was dispensed using a multichannel pipette and incubated for 10 min at room temperature. 100 µl/well of stop solution was dispensed, taking care to avoid dispensing of bubbles. Finally, it was read at 405 nm (OIE, 2004).

Data analysis

The result was analyzed in relation to location, agro ecology, species, sex and mode of service. Data were stored in Microsoft Excel spread sheet. The data were edited, coded and transferred into intercool Stata (2009) version 7.0 (Stata Corporation, College Station, Texas, USA) for descriptive and analytical statistical univariate logistic regression analysis to look for identification of hypothesized risk factors in line with the result.

RESULTS

Overall prevalence

A total of 230 equines sera were examined by blocking ELISA technique. Out of which 54 equines were found to be seropositive which made the overall prevalence to be 23.47%.

Prevalence of AHS based on districts

An attempt was made to see the influence of location on the prevalence of AHS and accordingly, the prevalence of 52.72, 10.47 and 17.98% were found in Dale, Hula and Wonsho, respectively, in difference between Dale and other districts were found to be statistically significant ($P < 0.05$) between the three locations (Table 1).

Prevalence of AHS based on agro ecology

AHS seropositivity was also assessed in relation to agro ecology and the prevalence of midland was 52.72% while it was 14.28% in the highland.

The difference between agro-ecology was statistically significant ($P < 0.05$) (Table 2).

Prevalence of AHS based on species

The prevalence of AHS was 46.97, 13.66 and 33.33% in donkeys, horses and mules, respectively. There was statistically significant difference among donkeys, horses and mules ($P < 0.05$) (Table 3).

Prevalence of AHS based on sex

The prevalence of AHS encountered in males were 28.66% while the correspondence value in female was 10.61% and this difference was seen to be significant statistically ($P < 0.05$) (Table 4).

Prevalence of AHS based on mode of service

The prevalence of AHS infection among pack horses were 48.21%, while that of cart and race and pack were found to be 37.50% and that of cart and pack were 50% and statistically significant difference was observed between pack and combination of race and pack. Comparison of cart, and cart and pack need to be reserved as the number is not comparable (Table 5).

Table 2. Prevalence of AHS versus agro ecology.

Agro ecology	Number of equines examined	Number of equines seropositive	Prevalence (%) 95% CI
Midland	55	29	52.72 (39.01-66.35)
Highland	175	25	14.28 (9.05-19.52)
Total	230	54	23.47

Table 3. Prevalence of AHS in different species of equines.

Species	Number tested	Number seropositive	Prevalence (%) 95%CI
Donkey	66	31	46.97 (34.61-59.33)
Horse	161	22	13.66 (8.30-19.03)
Mule	3	1	33.33 (0.00-1.77)
Total	230	54	23.47

Table 3. Prevalence of AHS in different species of equines.

Species	Number tested	Number seropositive	Prevalence (%) 95%CI
Donkey	66	31	46.97 (34.61-59.33)
Horse	161	22	13.66 (8.30-19.03)
Mule	3	1	33.33 (0.00-1.77)
Total	230	54	23.47

Table 4. Prevalence of AHS in relation to sex.

Sex	Number tested	Number seropositive	Prevalence (%) 95%CI
Male	164	47	28.66 (21.67-35.65)
Female	66	7	10.61 (2.98-18.23)

Table 5. Prevalence of AHS based on mode of service.

Mode of service	Number tested	Number seropositive	Prevalence (%) 95%CI
Pack	56	27	48.21 (34.71-61.72)
Cart	8	3	37.5 (08.07)
Race and pack	163	22	13.5 (8.20-17.80)
Cart and pack	2	1	50% (0.00-685.3)
Total	230	53	23.47

DISCUSSION

This study revealed the overall prevalence of the three woredas' of Sidama zone, Hawassa, Ethiopia. The present overall prevalence report almost coincides with report of 25% prevalence in selected districts of Jimma zone (Molalegn et al., 2010) but it is not similar to the findings of Demissie (2013) in southern Ethiopia, who

reported 33% prevalence. This might be attributed to geographical variation.

In this study, the prevalence difference observed between Dale and other districts (Wossho and Hula) were statistically significant ($P < 0.05$). Similarly, the difference observed between midland and highland were also statistically significant ($P < 0.05$). In this study, Dale was taken to be representative of midland, and Wonsho

and Hula for highland. Therefore, the difference observed among districts is confounder, while agro-ecology (midland and highland) is an important factor. Ecology indicates the vector population is possibly manifested by prevalence. Hence, it is suggested that the prevalence in midland could be due to high density of vector population as the ecology favor its breeding (Ende et al., 2011; Tesfaye et al., 2013).

High prevalence was observed in donkeys as compared to horses and mules. The species comparison was made between donkeys and horses alone and the prevalence in donkeys was higher than in horses. The difference was statistically significant ($P < 0.05$). This is contrary to the literature that donkeys and mules are resistant (Alemayehu and Benti, 2009). The deviation could be due to high exposure rate of donkeys which are usually in midland area of Dale.

It is also indicated that there was statistically significant difference ($P < 0.05$) between sex and higher prevalence in male than in female. One of the probable reason was that most of the samples of midland were donkeys and of the male sex. This difference resulted from agro ecological variation rather than sex.

The study further revealed that there was lower prevalence in combined mode of service of race and pack than in pack. Horses are used in combined mode service of race and pack. Donkey is mainly used either as pack or cart. Most of the samples of horses were taken from highland and donkeys from midland land. The difference of the prevalence of AHS in the mode of service is not due to the mode of service they used, but the difference results from the distinct variation between the agro ecology from where the samples were taken. Comparison of cart, and cart and pack was not done because the numbers were not comparable.

The present finding showed that the hypothesized risk factors and agro-ecology is the predominant risk for the prevalence of African Horse sickness in the study area. Therefore, regular vaccination with potent vaccine in line with the disease epidemiology, awareness of the public on the disease epidemiology and further investigations should be done to identify the various risk factors.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Acetylcholinesterase, glucose and total protein concentration in the brain regions of West African dwarf goats fed dietary aflatoxin

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A study was carried out on the effect of varied levels of dietary aflatoxin on acetylcholinesterase, glucose and total protein concentration in the brain regions of West African Dwarf goats. 20 West African Dwarf goats of about 5-6 months old were used for the trial and they were randomly allotted to four dietary treatments containing 0 (control), 50, 100 and 150 µg aflatoxin/kg diet. The animals were housed individually for the feeding trial in a completely randomised designed and the experiment lasted 12 weeks. At the end of the feeding trial, the animals were sacrificed and brain dissected into different regions. The different regions of the brain studied were medulla oblongata, amygdala, hippocampus, cerebral cortex, mid-brain, cerebellum, pons varoli and hypothalamus. Samples were collected from these regions and homogenised to determine acetylcholinesterase, glucose and total protein concentrations. Result showed that the acetylcholinesterase activity in the brain regions was not significantly influenced by the dietary aflatoxin among the treatments. Glucose concentration was significantly ($p < 0.05$) higher in the hypothalamus of animals fed 50 µg aflatoxin/kg and control diet than those fed 100 and 150 µg aflatoxin/kg. Total protein concentration in the medullar oblongata and the hypothalamic regions of the brain in animals fed 150 ppb was significantly ($p < 0.05$) higher than those on the control diet. The pH of the medulla oblongata, amygdala, hippocampus, cerebral cortex were significantly ($p < 0.05$) higher in goats fed 150 mg/kg than those fed the control diet. However, pH was not significant in the mid brain, cerebellum, pons varoli and hypothalamus among the treatments. This study suggests that dietary aflatoxin up to 100 ppb reduced glucose concentration in the hypothalamus and total protein in the medulla oblongata region of the brain with tendency to impair brain function.

Key words: Aflatoxin, WAD goat brain, acetylcholinesterase, total protein.

INTRODUCTION

Aflatoxins are an important group of mycotoxin produced by the fungi *Aspergillus flavus*, *A. parasiticus* and *A. nomius* (Diaz et al., 2008). Other species of *Aspergillus*

such as *A. bombycis*, *A. ochraceoroseus* and *A. pseudotamari* also produce aflatoxins (Bennett and Klich, 2003). On world-wide scale, the aflatoxins are found in

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stored food commodities and oil seeds such as corn, peanuts, cottonseed, rice, wheat, oats, barley, sorghum, millet, sweet potatoes, potatoes, sesame, cacao beans, almonds, etc., which on consumption pose health hazards to animals, including aquaculture species of fish, and humans (Abdel-Wahab et al., 2008). The toxin is highly oxygenated, heterocyclic, difuranocoumarin compounds that could be present in human foods and animal feedstuffs. Health effects occur in fish, companion animals, livestock, poultry and humans because aflatoxins are potent hepatotoxins, immunosuppressant, mutagens, carcinogens and teratogens.

In the tropical regions, where the climatic conditions favour luxurious growth of *Aspergillus* spp, people rely on commodities such as cereals, oilseeds, spices, tree nuts, milk, meat and dried fruits that are potentially contaminated by aflatoxins (Strosnider et al., 2006). Animals are predominantly affected by the *Aspergillus* spp, metabolite through ingestion of contaminated diet. Aflatoxin is metabolized by cytochrome p450 group of enzymes in the liver, where it is converted to many metabolic products like aflatoxin 8, 9 epoxide, aflatoxicol, aflatoxin Q1, aflatoxin P1, and aflatoxin M1, depending on the genetic predisposition of the species. The amount of aflatoxin 8, 9 epoxide metabolite decides the species susceptibility as this can induce mutations by intercalating into DNA, by forming adduct with guanine moiety in the DNA (Smela et al., 2001).

There is currently lack of information on the aflatoxin effect on the brain of West African Dwarf (WAD) goats. The brain also operates as central control of movement, balance, memory, thought and emotion in the body of an animal (Taylor, 1998). Knowledge of the biochemical composition of the brain is therefore important. Some reports on the biochemical characteristics of the brains of farm animals have been documented, most especially for boars (Egbunike, 1981), pigs (Adejumo and Egbunike, 2001a) and rabbit (Bitto, 2008) but not in relation to aflatoxin effect.

METHODOLOGY

Experimental location and materials

This study was approved by our institutional committee on the care and use of animals for experiment. The experiment was carried out at the small ruminant unit of the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. It is located at latitude 70° 20' N and longitude 40° 50' E. It is 200 m above sea level with average day time temperature of 23-27°C and relative humidity of 80-85%.

Clean maize grains were purchased from local market in Ibadan metropolis and sorted. Damaged, coloured and bad kernels were removed and disposed. Other ingredients used for the feeds were purchased from Kesmac Feed and Agric Consult feed mill, opposite University of Ibadan second gate, Ibadan.

Aflatoxin contaminated maize grains

Maize grain served as the aflatoxin carrier in the diets used for this

study. The maize grains used for the experiment was inoculated with toxigenic strain *Aspergillus flavus* predominant in Nigeria. This culturing and inoculation was done at the Plant Pathology Unit, International Institute of Tropical Agriculture, Ibadan, Nigeria. The concentration in the maize grains and the diet was determined as described by Suhagia et al. (2006).

Experimental animals

Total of 20 WAD bucks of 5 months old were purchased for the experiment. The weights of the animals ranged from 7-9 kg. The animals were acclimatized for 28 days at the experimental site for physiological adjustment to feed and environment. Concentrate diet and *Gliricidia* leaves were fed, and fresh water were provided for the animals throughout the experiment. The animals were vaccinated against peste des pesti ruminants and treated with ivomectin injection against endo and ecto-parasites. Other routine management practices were carried out during the experimental duration.

Experimental diets

Four diets were formulated to meet the nutrient requirement of the animals. Diet 1 which is the control in the experiment and contained cleaned maize without aflatoxin contamination. The contaminated maize grains were used in substitution for the uninfected maize grains to vary the concentration of aflatoxin among diets 2, 3 and 4. All diets were iso-nitrogenous (12.9% CP) and isocaloric (3.57kcal/g DE).

Treatment Layout

Diet 1: Control diet without aflatoxin
 Diet 2: Diet containing 50 ppb aflatoxin
 Diet 3: Diet containing 100 ppb aflatoxin
 Diet 4: Diet containing 150 ppb aflatoxin

Feeding trial

At the end of the acclimatisation period, 20 bucks were weighed and allotted randomly into four treatments such that each treatment has 5 animals housed individually in a completely randomised design. Dietary treatments 1, 2, 3 and 4 were represented with T1, T2, T3 and T4, respectively.

Dietary treatments were offered to the respective animals twice daily at 8.00 a.m. and 12.30 p.m. with their respective diets ad libitum. Feed supply was adequate and responsively supply as the bucks weight changes, since feed consumption would be expected to change with body weight. The experiment lasted for 12 wk, during which animals were fed concentrate as supplement to *gliricidia sepium* in ratio 2:3 of their body weight.

Brain regions assessment

At the end of the feeding trials the animals were sacrificed, head dissected to harvest the brain and the brain was separated into regions samples were taken from the following regions: amygdala, cerebellum, hypothalamus, hippocampus, pons varoli, mid-brain and medulla oblongata.

Total proteins concentration

The total protein concentrations in the regions were evaluated using

Table 1. Acetylcholinesterase (AChE, $\mu\text{mol/g/min}$) activity in the brain regions of West African dwarf bucks fed varied levels of dietary aflatoxins (Mean \pm SD).

Parameters	T1 (0 ppb)	T2 (50 ppb)	T3 (100 ppb)	T4 (150 ppb)
Medulla oblongata	0.85 \pm 0.85	0.29 \pm 0.04	0.51 \pm 0.16	0.42 \pm 0.34
Amygdala	0.57 \pm 0.83	0.25 \pm 0.17	0.51 \pm 0.16	0.22 \pm 0.10
Hippocampus	0.32 \pm 0.06	0.24 \pm 0.12	0.26 \pm 0.11	0.29 \pm 0.21
Cerebral cortex	0.65 \pm 0.53	0.34 \pm 0.23	0.32 \pm 0.26	0.16 \pm 0.14
Mid brain	0.38 \pm 0.32	0.22 \pm 0.11	0.69 \pm 0.64	0.81 \pm 0.72
Cerebellum	0.31 \pm 0.02	0.28 \pm 0.03	0.49 \pm 0.41	0.29 \pm 0.26
Pons varolii	0.28 \pm 0.12	0.42 \pm 0.25	0.66 \pm 0.95	0.25 \pm 0.16
Hypothalamus	0.19 \pm 0.09	0.29 \pm 0.02	1.08 \pm 0.90	0.48 \pm 0.39

the Biuret method as earlier reported by (Adejumo and Egbunike, 2001b). An automatic dispenser was used to measure 5 mL of Biuret reagent into a test tube and 10 μL of the homogenate will be added. The mixture was incubated at room temperature of about 25°C for 30 min. After incubation, the incubated mixture was poured into a clean cuvette. The side of the cuvette was thoroughly wiped

with tissue paper before it was placed inside spectrophotometer at wavelength of 540 nm to determine the protein concentration. The blank was used to standardize the spectrophotometer. The standard was prepared using 0.1 mL of total protein standard and 5 mL of Biuret reagent.

$$\text{Total protein concentration} = \text{Absorbance of homogenate} \times 6 \text{ g/dL} / \text{Absorbance of standard}$$

Glucose concentration

The glucose concentrations in the regions of the brain were evaluated using the method as earlier reported by (Bitto et al.,

2009). 10 μL of the homogenate was introduced into a test tube and 1 mL of the glucose reagent was added. The mixture was incubated at 37°C for 10 min. Some of the incubated mixture was poured in a clean cuvette and read at wavelength of 500 nm.

$$\text{Glucose concentration (mg/dL)} = \text{Absorbance of homogenate} \times 100 / \text{Absorbance of standard}$$

Acetylcholinesterase concentration

The regions were homogenized in 0.1 mL of phosphate buffer (pH 7.4) using Elvenjem glass homogeniser and latter assay for acetylcholinesterase (AChE) concentration was determined according to the calorimetric method as reported by Egbunike (1981) which measure the rate of hydrolysis and acetyl thiocholine iodine substrate to thiocholine and acetate using 5:5 dithiobis-2-nitrobenzoate (DTNB) as the colour reagent. 2.6 mL of the buffer was pipette inside the cuvette. 100 μL of DTBN and 0.4 mL of the homogenate were added and the mixture was placed inside the spectrophotometer and this was standardized to zero after which 20 μL of substrate was added. The initial absorbance was read and after 4 mins the final reading was taken at a wavelength of 405 nm.

Data analysis

Data obtained were subjected to analysis of variance at $p = 0.05$ and means were separated using Duncan's multiple range tests of SAS (1999). SAS/STAT® (version 8.0) (SAS Institute, Cary, North Carolina, United States).

RESULTS

Acetylcholinesterase activity in brain regions of WAD goats

The effect of aflatoxin on the AChE activity in the brain regions of WAD bucks is as shown in Table 1. The result obtained showed that there was no significant difference

the AChE concentration among the dietary treatments. The AChE in medulla oblongata, amygdala, hippocampus, cerebral cortex, mid-brain, cerebellum, pons varolis and hypothalamus was not significantly influenced by dietary aflatoxin among the treatments. The AChE observed in treated animals was not significantly different from the control.

Glucose concentration in the brain regions of WAD goats

The glucose concentration in the brain regions of WAD bucks fed varied levels of dietary aflatoxin is as shown in Table 2. The result obtained showed no significant difference in all the brain regions examined except hypothalamus among the treatments. It was observed that the glucose concentration in the hypothalamus of goats fed treatment 2 was not significantly different from those fed control diet. However, the value obtained for animals on T2 and T1 were significantly ($p < 0.05$) higher than those fed treatments 3 and 4. The glucose concentration in hypothalamus of goats fed treatment 3 was not significantly different from those fed treatment 4.

The pH and total protein concentration in brain region of WAD goats

The pH of the brain regions of WAD bucks fed dietary

Table 2. Glucose concentration (mg/dL) in the brain regions of West African dwarf bucks fed varied levels of dietary aflatoxins (Mean±SD).

Parameters	T1 (0 ppb)	T2 (50 ppb)	T3 (100 ppb)	T4 (150 ppb)
Medulla oblongata	16.93±8.19	10.27±4.36	6.26±5.89	12.06±1.35
Amygdala	9.23±9.13	17.29±1.03	9.06±8.20	13.10±1.35
Hippocampus	2.39±2.79	6.52±3.54	16.57±4.40	21.31±18.38
Cerebral cortex	11.43±7.80	10.77±3.41	15.03±7.03	16.75±8.39
Mid brain	20.57±10.92	3.70±4.67	19.08±21.60	17.40±10.12
Cerebellum	17.71±5.01	15.54±11.03	13.07±4.41	16.61±2.57
Pons varolii	10.71±3.58	12.76±10.55	18.02±7.68	11.47±3.40
Hypothalamus	16.39±8.55 ^a	12.33±8.32 ^a	9.78±2.07 ^b	8.55±4.30 ^b

ab = means along the same row with different superscripts are significantly ($p < 0.05$) different.

Table 3. The pH concentration of the brain regions of West African dwarf bucks fed varied levels of dietary aflatoxins (Mean ±SD).

Parameters	T1 (0 ppb)	T2 (50 ppb)	T3 (100 ppb)	T4 (150 ppb)
Medulla oblongata	7.30±0.17 ^b	7.43±0.04 ^{ab}	7.37±0.06 ^{ab}	7.51±0.01 ^a
Amygdala	7.33±0.06 ^b	7.42±0.06 ^{ab}	7.40±0.10 ^{ab}	7.50±0.02 ^a
Hippo-campus	7.37±0.56 ^b	7.44±0.03 ^{ab}	7.41±0.10 ^{ab}	7.53±0.03 ^a
Cerebral cortex	7.33±0.56 ^b	7.43±0.10 ^{ab}	7.39±0.12 ^{ab}	7.50±0.02 ^a
Mid brain	7.40±0.09	7.42±0.05	7.40±0.14	7.51±0.01
Cerebellum	7.33±0.06	7.43±0.15	7.41±0.11	7.50±0.03
Pons varolii	7.32±0.06	7.41±0.16	7.40±0.10	7.50±0.04
Hypothalamus	7.30±0.17	7.40±0.10	7.41±0.12	7.50±0.02

ab = means along the same row with different superscripts are significantly ($p < 0.05$) different.

aflatoxin is as shown in Table 3. No significant difference was observed in the pH of mid-brain, cerebellum, pons varoli, hypothalamus of the animals among the treatments. However, there was significant ($p < 0.05$) difference in the pH value of medulla oblongata, amygdala, hippocampus and cerebra among the treatments. The pH in these brain regions followed the same trend, and that of animal fed Treatment 4 were not significantly different from those fed treatments 2 and 3 but were significantly ($p < 0.05$) higher than those bucks on the control diet.

The total protein concentration within the brain regions of the bucks fed varied level of dietary aflatoxin is as shown in Table 4. The amygdala, hippocampus, cerebral cortex, cerebellum and pons varoli of the animal fed dietary aflatoxin were not significantly different among the treatments.

However, total protein concentration in medullary oblongata of the animal on treatment 1 was significantly ($p < 0.05$) lower than those on treatment 4 while those on treatments 2 and 3 were not significantly different from each other. Also, the hypothalamus of the animal fed treatment 4 which has the highest dose of aflatoxin recorded total protein concentration which was significantly ($p < 0.05$) higher than goats fed treatments

1, 2 and 3. However, the total protein in hypothalamus of goats fed treatments 2 and 3 was not significantly different from the control.

DISCUSSION

The ability of aflatoxin producing fungi (*Aspergillus flavus*) to grow on wide range of food and feed stuffs under certain condition constitutes a threat to both animals and human (Sayed and Abeer, 2013). In developing countries where food availability often times are considered before food safety, there is a lack of legislation on acceptable limits for aflatoxin and population are undoubtedly exposed to high amount of aflatoxin (Williams et al., 2004). The AChE activity that was not significantly different among the brain regions for all the treatments indicated that the inclusion of aflatoxin level up to 150 ppb does not affect normal synthesis and catabolism of neurotransmitters (AChE) which could presumably be tolerated by the animal since it does not affect any brain region biochemicals. This could probably be that goat has higher resistance to aflatoxicosis than sheep and cattle. Adejumo et al. (2005) reported from the study on sex differences in acetylcholinesterase activity in Red sokoto

Table 4. Total protein concentration (g/dL) of the brain regions of West African Dwarf bucks fed varied levels of dietary aflatoxins (Mean \pm SD).

Parameters	T1 (0 ppb)	T2 (50 ppb)	T3 (100 ppb)	T4 (150 ppb)
Medulla oblongata	1.24 \pm 0.02 ^b	1.59 \pm 0.89 ^{ab}	1.76 \pm 0.17 ^{ab}	2.59 \pm 0.60 ^a
Amygdala	0.66 \pm 0.14	1.68 \pm 0.78	1.11 \pm 0.40	1.34 \pm 0.56
Hippocampus	0.53 \pm 0.62	1.30 \pm 0.38	1.11 \pm 0.40	1.34 \pm 0.56
Cerebral cortex	1.48 \pm 0.24	1.73 \pm 0.25	1.27 \pm 0.25	2.12 \pm 0.28
Mid brain	1.05 \pm 0.32	1.32 \pm 0.35	1.02 \pm 0.21	1.53 \pm 0.86
Cerebellum	2.53 \pm 0.00	1.68 \pm 1.65	1.54 \pm 0.30	1.53 \pm 0.24
Pons varolii	1.27 \pm 0.09	0.68 \pm 0.05	1.28 \pm 0.83	0.66 \pm 0.47
Hypothalamus	1.35 \pm 0.22 ^b	2.00 \pm 0.50 ^b	1.54 \pm 0.18 ^b	2.66 \pm 0.10 ^a

ab = means along the same row with different superscripts are significantly ($p < 0.05$) different.

bucks and does that the AChE maintains its characteristically low concentration in Red sokoto bucks brain. However, AChE concentration was highest in the mid brain, medulla oblongata, hippocampus of pig brain (Adejumo and Egbunike, 2002).

The lack of micro doses of dietary aflatoxin effect on the AChE activity in the cerebellum may be due to its involvement in locomotion and muscular activities which are maintained in the animal up till old age (Adejumo and Egbunike, 2004). In addition, the cerebellum itself is characterised by typically low AChE activities (Adejumo and Egbunike, 2002). The normal level of AChE reported for Red Sokoto goats was 4.32 \pm 0.36 μ mol/g/min (Adejumo and Egbunike, 2002).

The significant difference that was observed in the protein concentration in medulla oblongata and hypothalamus could be due to change in protein synthesis or metabolism and could be attributed to under nutrition or poor nutrient utilization as reported in these same animals (Ewuola et al., 2013). Aflatoxin has been reported to binds and interferes with enzymes and substrates that are needed in the initiation, transcription and translation process involved in protein synthesis. This may also be indicative of aflatoxin effect on the brain development as protein in the brain is important for it functions such as repair of worn-out tissues for growth, muscles development and it also binds to some minerals to ensure bioavailability of minerals for proper utilisation (Adejumo et al., 2005).

The total protein levels in the brain regions in the study were generally higher than the values reported for the brain regions of male porcine by Adejumo and Egbunike (2001, 2001a) and Bitto (2008) for rabbit bucks. This disparity may be due to species differences in biochemical characteristics of brain regions. Also, total proteins in the brain undergo major changes during development (Tucek et al., 1990) and such changes have been found to be unaffected by genetic or species effects amongst some ruminant (Adejumo et al., 2005).

There were no significant differences in glucose concentration of the different brain regions, except

hypothalamus. The significant difference in the hypothalamus may be due to its involvement in glucose transport, energy production and glycogenesis which was also observed by Sayed and Abeer (2013) male Sprague rat fed aflatoxin. It could also indicate that WAD bucks may have increased activities in their brain with respect to hormone secretion (Taylor et al., 1998) since hypothalamus is involved in the release of gonadotropin releasing hormones in the brain.

The glucose concentration in the hypothalamus region of animal fed 100 and 150 μ g/kg aflatoxin significantly lower than those fed 50 μ g/kg aflatoxin and the control diet could be an indication of hypothalamic hypoglycaemia probably induced by the toxin. This implies that glucose is low, psychological process requiring mental effort may be impaired. The result corroborates the finding of Ikegwuonu (1983) who reported that nerves tissue requirement for glucose molecules were reduced during aflatoxicosis. Glucose has earlier been reported to be the obligatory energy substrate for fuelling brain and it is entirely oxidised to carbon iv oxide and water for optimal use (Magistretti et al., 1999; Kong et al., 2002).

The alteration in the pH of medulla oblongata, amygdala, hippocampus and cerebral cortex among the treatments could be attributed to treatment effect induced by the toxin. However the observe values across the treatments were within the physiological range of 7.2-7.5 reported by Bergmeyer (1974).

Aflatoxin does not have any significant effect on the brain pH, values obtained ranges from 7.3-7.5 in all the dietary treatments. The AChE is reported to be optimally active within a pH range of 7.2-8.5 (Bergmeyer, 1974). At a level beyond this range, AChE is inactivated (Bergmeyer, 1974), although WAD goats have been adjudged to be tolerable to aflatoxin.

CONCLUSION

Based on the results of this study, the acetylcholinesterase

activity and total protein concentration for the treatments were not influence across the brain regions by the micro doses of aflatoxin up to 150 µg/kg. However, the animals on 100 and 150 µg/kg suffer hypothalamic hypoglycaemic condition in the brain which may impair psychological process affect mental effort like coordination. This study suggests that dietary aflatoxin above 50 ppb reduced glucose concentration in the hypothalamus and total protein in the medulla oblongata region of the brain.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Seroprevalence of chlamydial abortion and Q fever in ewes aborted in the North-West of Algeria

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Very little information is available in Algeria on Q fever and chlamydial abortion sheep, two zoonosis caused by *Coxiella burnetii* and *Chlamydophila abortus* and their main reservoirs are domestic ruminants. This study aimed at investigating the seroprevalence of these two diseases in sheep flocks from six Daira (Telagh, Tanira, Moulay Slissen, Marhoum, Ras Elma and Merine). A serological survey was conducted in 39 flocks with a history of abortions, which were classified by size. A total 180 sera were collected from the aborted ewes. Q fever indirect ELISA kit and *C. abortus* indirect ELISA kit (ID Screen[®]) kits were used to know the percent prevalence in sheep. The results showed that 28% (N = 50/180) of sheep were seropositive for Q fever and 31% (N = 55/180) of sheep were seropositive for chlamydial abortion. Twenty eight herds (72%) showed at least one seropositive animal for Q fever and 29 herds (74%) showed at least one seropositive animal for chlamydial abortion. Larger herds led to more infected herds of small and medium for Q fever. These results showed that infection with Q fever and chlamydial abortion were common in the study area, therefore encouraging efforts are needed to propose measures to reduce the spread and zoonotic risk.

Key words: Q fever, chlamydial abortion, seroprevalence, enzyme-linked immunosorbent assay (ELISA), zoonosis.

INTRODUCTION

Chlamydial abortion and Q fever are two zoonoses caused by two small obligate intracellular Gram-negative bacteria, *Chlamydophila abortus* and *Coxiella burnetii* (Maurin and Raoult, 1999; Rodolakis, 2006; Aitken and Longbottom, 2007) which grow in the cytoplasm of

eukaryotic cells. They are the most important causes of reproductive failure in sheep and goats (Berri et al., 2001, 2005; Arricau-Bouvery and Rodolakis, 2005). These infections cause abortion, stillbirth, delivery of weak offspring and infertility in the small ruminant (Rodolakis et

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al., 2004; Agerholm, 2013). The losses caused by these two agents evaluated on several levels. In economic terms, the non-sale of the product (lamb or goat), the non-renewal of young breeding (antenaïse or goat) and decreased milk production (dairy farming) are the most negative impact on the scale of livestock. In health terms, the main concerns are the risk of contamination of several lots on livestock, as well as professional zoonotic transmission. Both agents were the subject of increasing research from the years 2002 in small ruminants, due to their proven implication in human focus (Wallenstein et al., 2010).

The present study attempted to investigate the prevalence of chlamydial abortion and Q fever at the level of district Sidi Belabbes through sero-prevalence studies in flocks that have experienced abortions and correlate its possible association with managerial (flock size, region, type of farming, and contact with other flocks) risk factors. In conjunction, the study try to clarify the interpretation of complementary investigations required by veterinary practitioners by confirming diagnostic tools available for both agents searched.

MATERIALS AND METHODS

Animals and blood sampling

This study was carried out from September to December 2013 (season of lambing); this study was based on the declaration of 1, 2 or 5 abortions on a period less than or equal to 4 weeks as equivalent to an abortion episode. While those with two reported abortions at 5 weeks of interval were eliminated from this study.

From 39 sheep flocks, a total of 180 blood samples were collected by jugular venous puncture in 4 ml sterile vacutainer tubes using Tubes BD Vacutainer® secs "BD, France" from aborted ewes aged between 1 and 6 years. After storage at room temperature for 1 h, blood samples were centrifuged at 3000 rpm for 5 min at room temperature. Sera were carefully harvested and stored at -20°C until assayed. Selected flocks ranged in size from 42 to 450 sheep. Flocks sizes were <100, 100-200 and >200 sheep for 6, 22 and 11 flocks, respectively, in order to establish the sero-prevalence of chlamydial abortion and Q fever via an indirect diagnosis. All flocks visited only once, no vaccine against chlamydial abortion or Q fever used.

Serological techniques

For the detection of antibodies against *C. burnetii* and *C. abortus*, two different ELISAs were used. For Q fever, indirect ELISA kit and *C. abortus* indirect ELISA kit (ID Screen® France) were used. Positive and negative control provided by the manufacturer and an internal positive laboratory reference was included in each test. Results were expressed as a percentage of the optical density reading of the test sample (% OD) calculated as $\% = 100 \times \text{OD sample} / \text{OD positive control}$. Recommended readings OD%<40 as negative, OD%>40, OD%<50 as doubtful, OD%>50<80% as positive and OD>80 highly positive for *C. burnetii*, and OD%<50 as negative, OD%>50<60 as doubtful, and OD%>60 as positive for *C. abortus*.

Statistical analysis

All data were entered and validated using a Microsoft Excel package. To bring out the association between a supposed risk factor and the disease, the odds ratio (OR) and relative risk (RR) were calculated.

The odds ratio is the probability of having the disease according to the presence or absence of risk factors and allows for addition to the degree of significance of the association, the direction and strength of the association.

RESULTS

According to the experimental design, all 39 flocks studied had a history of abortions and stillbirths, 180 aborted ewes belonging to these 39 flocks were examined for antibodies against *C. abortus* and *C. Burnetii*. The seropositivity results towards for these two bacteria obtained in aborted sheep at individual and flock levels were summarized in the Table 1.

At the farm level, 74% (29/39, 95% CI: 58 to 86) of farms had at least one seropositive sheep to *C. abortus* and 72% (28/39, 95% CI: 55-84) of farms had at least one seropositive sheep to *C. burnetii*. The seroprevalence of *C. abortus* infection in ewes is not associated ($P > 0.05$) with the three flocks size groups, it was the same for *C burnetii*. The seroprevalence rate ranged from 29 to 88% and from 45 to 95% for *C. abortus* and *C burnetii*, respectively.

Overall, the sheep level seroprevalence was 31% (55/180, 95% CI: 23, 92 -37, 84) for *C. abortus* and was 28% (50/180, 95% CI: 21, 37 -34, 93) for *C. burnetii*. In sheep level, there was significant difference ($P < 0.05$) between the seroprevalence of chlamydial infection and the location (Table 2). The highest prevalence rate (46.67%) of chlamydial infection was observed in Telagh area, while the lowest rate (17, 65%) was observed in Tanira area.

DISCUSSION

Exposure of sheep to *C. abortus* and *C. burnetii* was evaluated by testing for the presence of antibodies with an indirect ELISA test. The detected antibodies in this study imply a natural response to exposure to the microorganisms because there is no vaccination program against ruminant chlamydiosis or Q fever in Algeria before. The survey design provided data on seroprevalence at the flock and the animal level in this area. Results of the present study revealed an animal-level seroprevalence to *C. abortus* of 31% and to *C. burnetii* of 28% in Sidi Bel Abbes region, Algeria. This figure is higher than that reported from other Algerians regions (Khaled et al., 2016; Hireche et al., 2014;

Table 1. Seroprevalence of *Chlamydophila abortus* and *Coxiella burnetii* infection in sheep according to flock size in Wilaya of SIDI BEL ABBES west of Algeria (2013).

Parameter	Flock size			Total	
	<100	[100 - 200]	>200		
Number of flocks	6	22	11	39	
Aborted sheep	27	178	153	358	
Aborted sheep taken	17	90	73	180	
Chlamydial abortion	Number of seropositives sheeps ^a (%)	7/17 (41%)	34/90 (38%)	14/73 (19%)	55 (31%)
	Number of seropositives flocks ^b (%)	5/6 (83%)	18/22 (82%)	6/11 (56%)	29 (74%)
	Range of seropositives flocks (%)	57%-88%	60%-94%	29%-83%	58%-86%
Q fever	Number of seropositives sheeps ^a (%)	6/17 (35%)	31/90 (34%)	13/73 (18%)	50 (28%)
	Number of seropositives flocks ^b (%)	3/6 (50%)	15/22 (68%)	10/11 (91%)	28 (72%)
	Range of seropositives flocks (%)	45%-88%	45%-86%	57%-94%	55%-85%

^aAnimal is considered positive if serum is positive (%DO > 40 by ELISA). ^bFlock is considered positive if at least one animal serum is positive. * χ^2 test: $p < 0.05$.

Table 2. Seroprevalence of Q fever and chlamydial abortion depending on the region.

Area	Sera	Sera (+) for FQ	Seroprevalence (%)	Sera (+) for CH	Seroprevalence (%)
Telagh	30	7	23.23	14	46.67*
Ras Elma	30	7	23.23	15	50.00
Tanira	34	17	50	6	17.65*
M.slissen	40	8	20	9	22.50
Merine	27	8	29.63	7	25.93
Marhoum	19	3	15.79	4	21.05
Total	180	50	27.78	55	30.56

Sera (+) for QF: Sera positive for Q fever ; Sera (+) for CH: sera positives for chlamydial abortion. *Significant difference ($P < 0.05$) between areas by chi-square test.

Merdja et al., 2014). Also, several authors had previously reported high prevalence in Maghreb countries such as Morocco (El Jai et al., 2003), Tunisia (Russo et al., 2005), Egypt (Abdel-Moein and Hamza, 2017) and in world countries such as Turkey (Kennerman et al., 2010), Italy (Francesca et al., 2016), Slovakia (Trävnicsek et al., 2001), Spain (Mainar-Jaime et al., 1998) and Jordan (Al-Qudah et al., 2004). The overall seroprevalence rate at the flock level in our survey was 74% to chlamydial abortion and 72% to Q fever. These rates are higher than those reported by Francesca Rizzo et al. (2016) in flocks 38% in Italy and Angela et al. (2012) with 28% in Germany. However, due to numerous parameters such as differences in study design and inclusion criteria (e.g. high abortion rates), flock size and management, prevalence of other abortifacient agents (e.g. Brucellae, Salmonellae, Toxoplasma, Chlamydia, Campylobacter) it is virtually impossible to compare the present study's prevalence findings with the aforementioned studies

(Masala et al., 2004).

A higher prevalence rate was revealed for flocks with more than 200 animals compared with that of small flocks (91 and 50%, respectively) for Q fever, while, no significant correlation was revealed between flock size and the rate of seroprevalence for chlamydial abortion. The difference rate of seroprevalence revealed for the flock high size for Q fever might be that due to animal overcrowding in livestock buildings. Also may be related to the high number of lambing at lambing season, which increases the total population at risk and, subsequently, the risk of pathogen introduction and transmission, where high density may influence animal welfare and the occurrence of infectious diseases. The study showed that there is a significant difference ($P < 0.05$) between chlamydial infection in sheep and areas of northwest of Algeria (Table 2). Tanira and Ras Elma areas having the highest rate of chlamydial infection in sheep may be explained by the behavior of these species breeding in

these sites (frequency of herding group belonging to several farmers in the same village). These factors favor the rapid spread of infection.

Conclusions

The geographic distribution of *C. burnetii* and *C. abortus* indicate that both pathogens are present throughout the district of Sidi Bel Abbas. The highest percentage of positive samples was found for chlamydial abortion in Ras Elma (50%), and for Q fever in Tanira (50%). It seems that abortions in sheep following infection with *C. burnetii* and *C. abortus* have a higher frequency, even in young animals. Q fever and chlamydial abortion are a public health problem in Algeria. To for better control both in animals and humans, veterinary and public health sector should strengthen their collaboration for the establishment of a national program to fight against the major zoonoses in general and against Q fever and chlamydial abortion in particular.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Prevalence of bovine trypanosomiasis in Gamogoffa Zone, Ouba Debrestahay District, Southern Ethiopia

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This cross-sectional study was conducted in Ouba Debre-Tsehay District, Gamogoffa Zone, Southern Ethiopia from October 2013 to July 2014 with the objectives of estimating the prevalence of bovine trypanosomiasis and to assess the possible risk factors. Blood samples were collected from 384 randomly selected cattle from purposefully selected peasant associations with consideration of different age groups and both sexes. Buffy coat method was employed for parasitological survey and packed cell volume (PCV) determination was done. From the examined animals, 58 (15.1%; 95% CI: 11.7-19.6) were positive for trypanosome infection. In the area, *Trypanosome congolense* and *Trypanosome vivax* were the two identified *Trypanosoma* species with proportion of 60.3 and 27.6% , respectively and 12.1% mixed infection. Trypanosomiasis was observed as 13.8% in males and 15.9% in females out of these, 4.9 (95% CI: 1.0-13.7), 11 (95% CI: 5.8-18.4) and 20.1% (95% CI: 14.9-26.1) were found in animals <1year, between 1 and 3 years and above 3 years of age, respectively. Significant difference was observed between <1 year and >3 years of age ($p<0.05$). Based on body condition category, 8 (95% CI: 4.7-13.8), 16 (95%CI: 10.2-23.5) and 26.1% (95%CI: 17.3-36.6) were good, medium and poor conditioned animals, respectively and there was a significant association between good and poor condition animals ($p<0.05$). The recorded overall mean PCV in parasitaemic and aparasitaemic animals was 20.4 and 25.6%, respectively; it significantly varied ($t=12.28$, $p = 0.00$). Out of the parasitaemic and aparasitaemic animals, 77.6 and 16.6% were anemic, respectively (with PCV < 24%).

Key words: Cattle, Ouba Debre-Tsehay, prevalence, *Trypanosoma*, trypanosomiasis.

INTRODUCTION

Cattle population of Ethiopia is estimated at about 49.3 million heads (CSA, 2009). Despite its huge population size, cattle productivity remains marginal due to various diseases, malnutrition and management constraints. Parasitism represents a major obstacle to the development of sub-sector of which trypanosomiasis plays a great role (FAO, 2005). Animal trypanosomiasis is

a protozoan parasitic disease of domestic animals resulting from infection with different *Trypanosoma* species transmitted by tsetse fly and other haematophagous flies, mechanically (Sharma et al., 2013). It is a chronic, debilitating disease, which occur in some 240,000 km² area of Ethiopia. About 10-14 million heads of cattle and a significant number of

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small ruminants and equines are under serious risk of contracting the disease of which 20,000 heads die every year (Solomon, 2006). Animals affected with trypanosomiasis become anaemic and weak; lose weight and have reduced productivity, and often, mortality rates are high due to pathology induced by trypanosomes (Urquhart et al., 1996; Bal et al., 2012).

In Ethiopia, there are five economically important animal *Trypanosoma* species; however, *T. congolense* is responsible for the most important form of animal trypanosomosis in domestic animals and also the most prevalent trypanosome species in tsetse-infested areas of the country (Leak, 1999).

Trypanosomosis in Africa is mainly restricted to areas in which the vector, tsetse fly species like *G. m. submorsitans*, *Glossina pallidipes*, *Glossina fuscipes fuscipes* and *Glossina tachinoides* can survive. These species of tsetse flies are distributed along the lowlands of western, southern and southwestern part of Ethiopia. The disease is also found outside the tsetse belt areas transmitted mechanically by biting flies of the genus *Tabanus*, *Haematopota*, *Chrysops* and *Stomoxys*. This type of transmission has caused the spread of *Trypanosoma evansi* and *Trypanosoma vivax*, outside tsetse infested areas (Abebe, 2005). In very acute infections with highly susceptible exotic animals, infection with *T. vivax* can also pass through the placenta and into the fetus in pregnant animals. As a result, some cows abort and some calves are born before birth time (Abebe and Jobre, 1996).

Trypanosoma congolense and *T. vivax* exert their effect mainly by causing severe anemia and mild to moderate organ damage. The onset and severity of the anemia is directly related to the appearance of the parasite in the blood and to the level of the parasitaemia. The rapid decline in the hemoglobin concentration, red blood cell number and PCV and the clear clinical sign of pallor of the mucus membrane reveals that the animal is infected with trypanosome (OIE, 2008).

Animal Trypanosomosis in and around Arbaminch has socio-economic impact due to debilitation and death of untreated animals and reduces production and productivity of affected animals (Waldeyes and Aboset, 1997); but the disease had not yet been assessed and there is no documented baseline data in Ouba Debre-Tsehay district. Therefore, this study was done with the objectives of estimating the prevalence of bovine trypanosomiasis, to identify the predominant species of trypanosomes and to estimate the risk factors for the occurrence of the disease in the study area.

MATERIALS AND METHODS

Study area

Ouba Debre-Tsehay district is found in southern Ethiopia at 549 km from Addis Ababa, and 285 km from Arbaminch. The altitude of the selected PAS varies from 1001 to 1600 m.a.s.l. The rainfall pattern

is bimodal; a short rainy season runs from March to May and long rainy season runs from June to September and the average annual rain fall ranges from 600 to 1800 mm. The mean temperature varies from 27- 40°C. The land is covered by different vegetation types mainly savanna grassland forest, and bush lands predominated by acacia tree. The district has a livestock population of 218732 cattle, 90670 sheep and goat, 3947 equines and 250600 poultry (MARDO, 2011).

Study population

The study population was local breed zebu cattle which are kept in traditional management system. The animals in the area mainly depend on communal grazing fields and crop residues as feed source and watering points are the tributaries of Lomat and Bezo Rivers which is infested with tsetse flies predominantly by *G. pallidipes* and *Tabanus* (Amenu et al., 2008).

Sampling method and sample size determination

A cross-sectional study using simple random sampling technique was employed to determine the prevalence of bovine trypanosomiasis in the study area. The 8 PAs (Shele-bune, Yela-shabo, Galada, Zeka –zelto, Hoshele- shambara, Shala-Tito-Tife, Beto and Bala) were selected purposively based on the availability of transportation and logistics as well as their agroecological representativeness for the district was considered. From each selected PA, the farmers as well as the study animals were selected randomly in each household. During sampling, PAs, age, sex and body condition score (BCS) of the animal were recorded. The body condition score was grouped into good, medium and poor conditioned animals based on the appearance of ribs and dorsal spines applied for zebu cattle (Nicholson and Butterworth, 1986). Age of the animal was estimated by dentition (De-lahunta and Habel, 1986) and owner's information. The desired sampling size was calculated according to the formula given by Thrusfield (2005) with the expected prevalence of 50%, 95% confidence level and 5% absolute desired precision; as a result, the maximum sample size (384) was taken.

Trypanosome survey

Parasitological and hematological techniques were employed to detect the trypanosome and determine blood PCV, respectively. Accordingly, blood samples were obtained by bleeding marginal ear veins of cattle using a sterile lancet and drawing the blood into the heparinized capillary tube up to 3/4th of the length. The collected blood was sealed with crystal sealant and centrifuged for about 5 min with 12,000 rpm. After centrifugation, the packed cell volume (PCV) level was measured using hematocrit capillary reader and the length of the packed red cells column was expressed as a percentage of the total volume of blood. Animals with PCV below 24% was designated to be anemic (OIE, 2008).

Blood smears were made via cutting the centrifuged blood containing capillary tube 1 mm above and below the buffy coat layer using a diamond tipped pencil so as to include plasma and red blood cell (RBC) in the blood smear. The blood then expanded onto the clean glass slide, mixed well and covered with a clean cover glass. Examination was done under 40x objective lenses and 10x eye piece magnification, and the parasites were identified based on their morphology and movement in wet film preparation (Radostits et al., 2007). When the presence of the parasite was determined, a small drop of blood from a micro-haematocrit

Table 1. Prevalence of bovine Trypanosomosis in Ouba Debre-Tsehay district and proportion of the disease in the eight PAs.

PAs	Number of animals examined	Trypanosoma species			Total positives (%)	(95% CI)	χ^2 (P-value)
		<i>T. congolense</i> (%)	<i>T. vivax</i> (%)	Mixed (%)			
Shele-bune	56	6(60)	3(30)	1(10)	10 (17.9)	8.9-30.4	4.1 (1.00)
Yela-shabo	49	4(50)	3(37.5)	1(12.5)	8 (16.3)	7.3-29.7	
Galada	48	4(57.1)	2(28.6)	1(14.3)	7 (14.6)	6.1-27.8	
Zeka –zelto	46	5(71.4)	1(14.3)	1(14.3)	7 (15.2)	6.3-28.9	
Hoshele- shambara	48	5(71.4)	2(28.6)	-	7 (14.6)	6.3-27.8	
Shala-Tito-Tife	51	5(62.5)	2(25)	1(12.5)	8 (15.7)	7.0-28.6	
Beto	42	2(40)	2(40)	1(20)	5 (11.9)	4.0-25.6	
Bala	44	4(66.7)	1(16.7)	1(16.7)	6 (13.6)	5.2-27.4	
Total	384	35(60.3%)	16(27.6%)	7(12.1%)	58(15.1%)	11.7-19.1	

capillary tube was applied to a clean slide and spread by using another clean slide at an angle of 45°. The smear was dried by moving it in the air and fixed for 2 min in methyl alcohol. The thin smear was flooded with Giemsa stain (1:10 solution) for 30 min. Excess stain was drained and washed by using distilled water. Then, it was allowed to dry and examined under the microscope (100x) oil immersion objective lens (OIE, 2008).

Data management and analysis

The collected raw data and the results of parasitological and hematological examination were entered into a Microsoft excel spread sheet. Then, the raw data was summarized using SPSS version 20.

The prevalence of trypanosome infection was calculated as the number of positive animals as examined by Giemsa stain of thin blood film and buffy coat method divided by the total number of animals examined at the particular time. Pearson's Chi-square (χ^2) was used to evaluate the association of different variables with the prevalence of trypanosome infection and independent t-test was used to compare the mean PCV value between parasitaemic and non parasitaemic animals. P-value less than 0.05 was considered as significant.

RESULTS AND DISCUSSION

This study show that the overall prevalence

was 15.1% (95% CI: 11.7-19.1) which is in agreement with the reports of Feyissa et al. (2011) in Humbo District, Southern Ethiopia, but higher than the reports of Ayana et al. (2012) and Achenef and Admas (2012) in Amhara region. The higher prevalence might be due to the presence of frequent tsetse fly challenge as a result of high density in the study area (Amenu et al., 2008).

T. congolense and *T. vivax* were the two trypanosome species identified. However, higher proportion of *T. congolense* (60.3%) was observed than *T. vivax* (27.6%) and also 12.1% mixed infection (Table 1) which is in agreement with the reports of Feyissa et al. (2011). This might be due to the absence of significant variation in vector density and agro-climatic difference (Dagnachew, 2004).

In the present study, higher prevalence was observed in females (15.9%) than males (13.8%) which is in agreement with the reports of Feyissa et al. (2011) who reported 15 and 13.7% in female and males, respectively but there was no significant difference ($P > 0.05$) (Table 2). This was inconsistent with the report of Abrham and Tesfaheywot (2012). The possible explanation for

relative increment of prevalence in female animals might be due to physiological differences (Torr et al., 2006).

Based on age category, 4.9, 11 and 20.1% prevalence was observed in animals less than one year, between one and three years and above three years of age, respectively which revealed significant variation ($P < 0.05$) (Table 2). This might be due to young animals slightly protected by maternal antibodies (Fimmen et al., 1999).

The overall prevalence of trypanosome infection was significantly associated with the body condition of the study animals ($P < 0.05$) (Table 2). The prevalence was higher in poor body condition score animals (26.1% [95%CI: 17.3-36.6]) than the medium (16% [95% CI: 10.2-23.5]) and good (8.5% [95%CI: 4.7-13.8]) score animals. This is comparable with the finding of Feyissa et al. (2011). This is because Trypanosomosis is a chronic wasting disease characterized by slow progressive loss of condition (Uilenberg, 1998).

In this study, out of the total animals examined, 25.8% were anemic having PCV $< 24\%$ and 74.2% were not anemic (PCV ≥ 24). On the other hand, out of the total 58 parasitaemic animals, 77.6%

Table 2. Prevalence of Trypanosomosis based on sex, age and body condition score of examined animals.

Variables	Number of examined	Number of positive (%)	(95% CI)	χ^2 (p-value)
Sex				
Male	145	20 (13.8%)	8.6-20.5	0.31 (0.6)
Female	239	38 (15.9%)	11.5-21.2	
Age				
≤1 year	61	3 (4.9%)	1-13.7	10.5 (0.005)
1-3 years	109	12 (11%)	5.8-18.4	
>3 years	214	43 (20.1%)	14.9-26.1	
BCS				
Good	165	14(8.5%)	4.7-13.8	14.1 (0.001)
Medium	131	21 (16%)	10.2-23.5	
Poor	88	23 (26.1%)	17.3-36.6	
Total	384	58 (15.1%)	11.7-19.1	

Table 3. Comparison of mean PCV between parasitaemic and aparasitaemic cattle.

Condition	Total examined	Number of PCV <24%	Number of PCV ≥24%	Mean PCV	[95% CI]	t-test (p-value)
Parasitaemic	58	45(77.6%)	13 (22.4%)	20.4	19.6-21.3	12.28 (0.001)
Aparasitaemic	326	54 (16.6%)	272(83.4%)	25.6	25.28-25.9	
Total	384	99(25.8%)	285 (74.2%)	24.8	24.5-25.2	

were anemic (PCV<24) and only 22.4% were not; whereas, from 326 aparasitaemic animals, only 16.6% were anemic (PCV<24) but 83.4% were not anemic. There was significant difference between the mean PCV values of parasitaemic and aparasitaemic animals ($t=12.28$, $p<0.05$) (Table 3). This lower PCV was reported in previous studies in different parts of the country (Nigatu, 2004; Abraham and Tesfaheywet, 2012).

CONCLUSION AND RECOMMENDATIONS

Trypanosomosis is an important disease and a potential threat to health and productivity of cattle in Ouba Debre-Tsehay district. The result revealed that *T. congolense* is the most prevalent species in the study area and the infections significantly affect the PCV values and body condition. Therefore, economical and environment friendly community based tsetse fly and typanosomosis control program should be designed and implemented in the area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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